Program/Abstract # 168
Elucidating mechanisms of left–right patterning in vertebrate embryos
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Recent interest in genetic determinants governing vertebrate organ laterality has yielded numerous genes, mutations, and pathways implicated in left–right (LR) asymmetry. We study mutations in the zebrafish that cause abnormal LR patterning in the developing embryo. Two such mutations are *flanders* and *midway*, which cause abnormal organ situs and earlier abnormal asymmetric gene expression. *flanders* embryos exhibit roughly equal proportions of *situs solitus*, *situs inversus*, and heterotaxic embryos. *flanders* also randomizes normally asymmetric *nodal* cassette expression in the lateral plate mesoderm (LPM). I am currently mapping the *flanders* mutation to a particular gene in the zebrafish genome. By contrast, *midway* embryos exhibit a high incidence of reversed liver and pancreas positions with mostly proper heart looping, and lack any expression of normally asymmetric LPM genes. I have mapped *midway* to chromosome 12 and found that it fails to complement the *schmalspur* allele of the transcription factor *FoxH1*, the transcriptional effector of Nodal signaling. I have confirmed that the *midway* lesion is distinct from that in the *schmalspur* allele and is not located in the *FoxH1* coding region. I am currently working to confirm *midway's* identity as a regulatory mutation in the *FoxH1* genomic sequence. Studying these mutants in further detail will help elucidate the mechanisms that establish LR asymmetry in the vertebrate embryo.

doi:10.1016/j.ydbio.2008.05.180

Program/Abstract # 169
Serum Amyloid A is required for hedgehog signaling in zebrafish morphogenesis
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Serum Amyloid A is an acute phase protein synthesized by the liver and found in serum which is produced in response to infection or trauma; it has been implicated in inflammation and cholesterol transport processes. SAA is highly conserved in vertebrates, but in mammals is a multi-copy gene while in the zebrafish SAA is a single-copy gene. Little is known about the in vivo function of SAA, though increased SAA levels is correlated with increased risk of cardiovascular disease. Surprisingly, for a gene implicated in inflammation and possibly immune function, SAA is expressed early in fish development. We have utilized morpholino anti-sense experiments to determine whether SAA functions in zebrafish development. Both splice-blocking and translation blocking SAA morpholinos produce morphogenic defects including small, close-set eyes, U-shaped somites and defective myoseptum, suggesting that SAA is required for the function of the hedgehog- (hh-) signaling system. These phenotypes are rescued by injection of mouse SAA mRNA. SAA is required for hh-mediated differentiation of the vasculature, somitic myofibers and neural tube. To determine whether hh-signaling is disrupted in SAA morphants, we performed ptc in situ hybridization. Ptc was expressed more broadly, but less intensely in SAA morphants, suggesting that the hh-morphogenic gradient was affected by SAA. We are investigating the shh gradient, shh protein expression, and cholesterol transport in SAA morphant fish.

doi:10.1016/j.ydbio.2008.05.181

Program/Abstract # 170
gadd45b is expressed at the determination front and is regulated by Fgf and RA signaling during zebrafish somitogenesis
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In vertebrates, somites form in an anterior to posterior wave, with new somites deriving from tissue generated by the growing tail bud. Morphological segment boundary formation is presaged by oscillating gene expression cycles; these “waves” of gene expression begin in the tail bud and move anteriorly into the presomitic mesoderm (PSM) until they encounter a “determination front” that stabilizes gene expression. In vertebrates, the opposing gradients of FGF/Wnt signaling and Retinoic Acid (RA) signaling are important for defining the position of the determination front. *gadd45b* expression overlaps with genes expressed at the level of the determination front and *gadd45b* expression is sensitive to perturbation of determination front Fgf and RA signaling gradients. We have shown that Fgf negatively regulates *gadd45b* expression and that exogenous RA increases the *gadd45b* expression domain. We are using *gadd45b* depletion to further understand the role of Gadd45b in patterning and somite differentiation.

doi:10.1016/j.ydbio.2008.05.182

Program/Abstract # 171
Coupling vs. noise: The rise and fall of synchrony in the segmentation clock
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The "segmentation clock" is thought to coordinate sequential segmentation of the body axis in vertebrate embryos. This clock comprises a multicellular genetic network of synchronized oscillators, coupled by intercellular Delta–Notch signaling. How this synchrony is established and how its loss determines the position of segmentation defects in Delta and Notch mutants are unknown. We analyzed the clock's synchrony dynamics by varying strength and timing of Notch coupling in zebra-fish embryos with techniques for quantitative perturbation of gene function using Morpholino and DAPT. We developed a physical theory based on coupled phase oscillators explaining the observed onset and rescue of segmentation defects, the clock's robustness against developmental noise, and a critical point beyond which synchrony decays. We conclude that synchrony among these genetic oscillators can be established by simultaneous initiation and self-organization and that the segmentation defect position is determined by the difference between coupling strength and noise.
Science (2007) 317: 1911.

doi:10.1016/j.ydbio.2008.05.183